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Bypass induced liver disease: an experimental study of the effect of post-operative protein supplementation and metronidazole therapy in an animal model

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Summary. An animal model of jejuno–ileal bypass (JIB) with post-operative weight loss and liver dysfunction was established in the rat. The role of a protein supplemented diet and post-operative metronidazole was investigated using this model. The use of a protein supplemented diet alone markedly reduced the detrimental effects of JIB. Although a beneficial effect was also noted with post-operative metronidazole, it was less marked and there appeared to be no additive benefit when both were used together. The results of this study would support the routine use of a protein enriched diet post-operatively in patients undergoing JIB.

Keywords: obesity surgery, animal model, liver dysfunction, dietary protein, metronidazole

Intestinal bypass operations have been shown to be effective in achieving weight loss in patients with morbid obesity (Hocking *et al.*1983; Scott *et al.* 1970). However the weight reduction has frequently been associated with serious side effects (Halverson *et al.* 1980; Hocking *et al.* 1983; O'Leary 1980). Abnormal liver function is clinically one of the most significant complications and is a common indication for bypass reversal (Brown *et al.* 1974; Halverson *et al.* 1980; Maxwell & McGouran 1982). As a result of this and other complications, jejuno–ileal bypass (JIB) can no longer be considered the operation of first choice in the surgical treatment of obesity (Griffen *et al.* 1977; Rucker *et al.* 1982). Nevertheless, some patients still require to be treated by bypass surgery, and a large population exists on whom the operation has been performed in the past. Therefore, the pathogenesis of the liver dysfunction remains both of practical and scientific interest.

A number of studies have been carried out to investigate liver dysfunction following bypass surgery. Two major aetiological factors have been proposed. The first related to bacterial overgrowth with consequent production of toxins affecting the liver (Hollenbeck *et al.* 1975; O'Leary *et al.* 1974; Sherr *et al.* 1974). The second concerns the nutritional status of the post-operative patient, implying that a relative protein deficiency state may be of primary importance (Moxley *et al.* 1974; Shizgal *et al.* 1979). To be of real clinical value, prevention of the liver dysfunction rather than treatment of the established disease is desirable. This study was

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therefore undertaken to examine the effect of protein supplementation and post-operative metronidazole therapy in a rat model of JIB, in which the liver dysfunction was a consistent feature.

Materials and methods

This study comprised two parts. The first was a pilot study to establish a suitable model of the JIB in the rat. The model was required to demonstrate consistent post-operative weight loss as seen in patients undergoing this procedure for morbid obesity, and also to show hepatic dysfunction that could be assessed by biochemical and histological parameters. For the pilot study 25 adult female Wistar rats (weight range 215–229 g) were randomly allocated into five equal groups. Following an overnight fast, all animals underwent surgery under pentobarbitone anaesthesia (6 mg/kg body weight i.p.). The experimental group of 20 rats all had a JIB. The jejunum was sectioned at 40 cm (Group A), 30 cm (Group B), 20 cm (Group C) and at 10 cm (Group D), below the pylorus, and in all animals the distal bowel was closed. Intestinal continuity was re-established by end-to-side jejuno–ileal anastomosis 4 cm from the ileo–caecal junction. Five rats serving as controls had division of the jejunum at the mid point, then a re-anastomosis end-to-end (Group E). All anastomoses were carried out using interrupted 6/0 polypropylene sutures and 2/0 silk was used for the abdominal closure. In the immediate 24 h post-operative period, all animals were restricted to liquid Vivonex alone and thereafter all returned to the standard laboratory solid pellet diet (41B, Dixon & Sons, Ware, Herts, UK). The rats were inspected daily and weighed weekly until the 8th week when all were killed and blood taken for liver function tests (LFTs) and livers fixed in 10% formalin for histological examination.

The body weight results of the pilot study are shown in Fig. 1. Group D satisfied the requirement of consistent post-operative weight loss with severe hypoproteinaemia and raised alkaline phosphatase. Histologically, the livers from Group D animals showed fatty changes which varied from severe deposition around the periportal region to diffuse involvement with mild fatty infiltration around the central vein. In a few livers, the fatty droplets were large with areas of focal necrosis. Portal cellular inflammation and mild ductular proliferation was

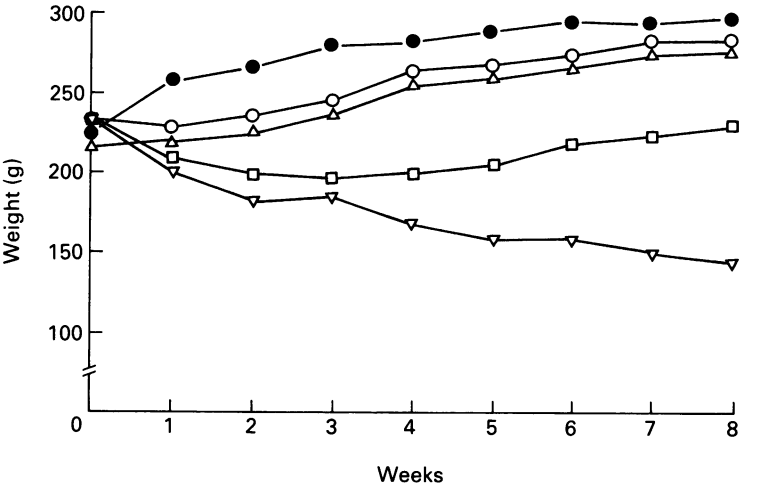

Fig. 1. Median weight curves for the five groups in the pilot study. ●, Controls; △, Group A; ○, Group B; □, Group C; ▽, Group D.

seen in several livers. The 10 cm to 4 cm JIB was therefore selected as a suitable model for the purpose of this study.

In the second and major part of this investigation, 50 adult female Wistar rats (weight range 200–230 g) were used. After an overnight fast, ten rats serving as controls underwent division and re-anastomosis of the bowel and the remaining 40 rats underwent a JIB as described above. On the second post-operative day the 10 control rats were randomly allocated into two equal groups. Group 1 (ND) received a standard laboratory diet; Group 2 (HPD) received a high protein diet which provided an additional 25% digestible crude protein (PRN, Dixon & Sons, Ware, Herts, UK). The 40 bypass rats were also randomly allocated into four equal groups. Group 3 (JIB/ND) were provided with standard laboratory diet; Group 4 (JIB/HPD) with high protein diet; Group 5 (JIB/ND+Met) with standard diet together with metronidazole in the drinking water to provide a dose 24 mg/kg/day calculated from the recommended paediatric dose for anaerobic infections (British National Formulary 1986); and Group 6 (JIB/HPD+Met) with high protein diet together with metronidazole in the same dosage as Group 5. Animals were inspected daily and weighed weekly and at the end of the 8th week, under ether anaesthesia, all animals were exsanguinated and subjected to full post mortem. The livers and other selected organs were removed whole and after fixation prepared for histological examination. Blood was sent to the routine chemical pathology laboratory for estimation of total protein, albumin, globulin, alkaline phosphatase, bilirubin, alanine and aspartate transaminase and gamma glutamyl transferase (Technicon, SMAC II). For both parts of the study the mortality was 0%.

All group data were expressed as medians and ranges and statistically compared using Wilcoxon's rank sum test for unpaired data and the Fisher Exact test.

Results

Body weight changes in the six groups are shown in Fig. 2. The two control groups fed on a normal or a protein supplemented diet gained weight and continued to do so at the

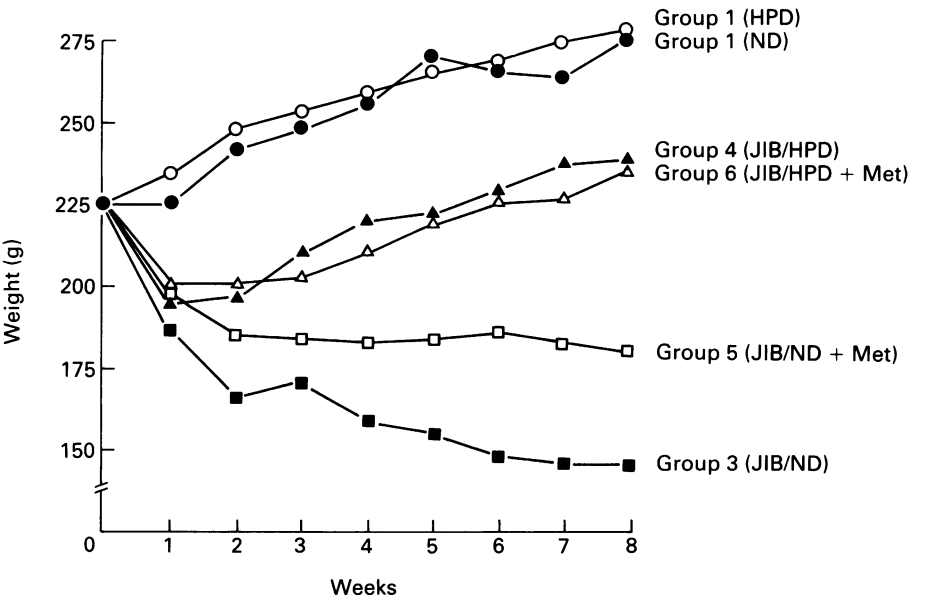

Fig. 2. Median weight curves for the control animals and the jejuno–ileal bypass Groups 3–6.

normal growth rate for animals of the size and age. All the rats in whom a JIB had been performed lost weight in the first post-operative week and thereafter the pattern of weight loss differed between the groups. Group 3 (JIB/ND) showed progressive weight loss throughout the study, though the rate of weight loss was greater in the first 4 weeks than in the second part of the experiment. Group 5 (JIB/ND+Met) showed an initial weight loss of 20% and then maintained a steady weight for the remainder of the study period. Groups 4 (JIB/HPD) and 6 (JIB/HPD+Met) showed a similar pattern of initial weight loss of 14% in the first 2 weeks, followed by a period of weight gain. At the end of the experiment there were no weight differences between the two control groups, nor between Groups 4 and 6. However, compared with the two control groups, there were significantly greater weight losses in all the JIB groups (P values <0.01). Comparisons between the JIB rats showed significantly greater weight losses in Groups 3 and 5 when compared to Groups 4 and 6 (P values <0.01) and in Group 3 rats when compared to Group 5 ($P<0.01$). In the subsequent section the data for the two control Groups 1 and 2 were considered together, forming a single control group of 10 rats.

The results for liver function tests for all the groups are detailed in Table 1. The lowest total protein values were seen in Group 3 (JIB/ND), being significantly lower than both Group 4 (JIB/HPD) and Group 6 (JIB/HPD+Met) (P values <0.01), but not different from Group 5 (JIB/ND+Met). The animals in Group 4 (JIB/HPD) had levels significantly higher than Group 5 (JIB/ND+Met) ($P<0.01$) but the levels observed in Group 4 were themselves significantly lower than Group 6 (JIB/HPD+Met) ($P<0.01$). Total protein levels in Group 5 (JIB/ND+Met) were significantly lower than in Group 6 (JIB/HPD+Met) ($P<0.01$). When compared to controls, Groups 3, 4 and 5 had significantly lower total plasma proteins (P

Table 1. Medians and ranges of liver function tests in the five experimental groups

Liver function tests	Groups 1 and 2 controls	Group 3 JIB/ND	Group 4 JIB/HPD	Group 5 JIB/ND+Met	Group 6 JIB/HPD+Met
Total protein g/l	62 (58–70)	42 (37–45)	53 (49–60)	43 (35–48)	63 (56–65)
Albumin g/l	35 (32–39)	24 (12–28)	29 (25–32)	25 (19–28)	33 (29–37)
Alkaline phosphatase iu/l	90 (54–144)	140 (89–211)	83 (47–155)	68 (52–215)	140 (60–160)
Total bilirubin μmol/l	2 (1–2)	2 (1–3)	2 (2–4)	1 (1–2)	2 (1–2)
Conjugated bilirubin μmol/l	0 (0–1)	0 (0–1)	0 (0–1)	1 (0–1)	1 (0–1)
Alanine transaminase iu/l	64 (53–182)	98 (35–146)	60 (39–126)	81 (55–267)	80 (42–185)
Aspartate transaminase iu/l	175 (95–253)	173 (98–354)	183 (80–214)	145 (131–239)	195 (158–405)
γ-glutamyl transferase iu/l	0	1 (0–1)	1 (0–1)	0	0

values < 0.01) whilst Group 6 did not differ. A similar pattern of differences between the groups was seen for the albumin data. Of the various liver enzymes assayed only alkaline phosphatase differed between the groups. Raised levels were noted in Groups 3 (JIB/ND) and 6 (JIB/HPD+Met), these being significantly higher than in any of the other three groups (P values < 0.01), which in themselves did not differ significantly. There were no differences between the groups with respect to alanine or aspartate transaminase, γ-glutamyl transferase or levels of total and conjugated bilirubin (Table 1).

Histologically, the livers from both control Groups 1 and 2 were normal. Livers from Group 3 animals (JIB/ND) showed the severest pathology, with gross fatty infiltration around the periportal region (Fig. 3) with some livers showing diffuse, generalised mild fatty infiltration. Mild portal inflammation and severe ductular proliferation was also seen (Fig. 4). Livers from Group 5 animals (JIB/ND+Met) had mild fatty infiltration around the periportal region with slight cellular infiltration. Whilst Group 4 (JIB/HPD) livers were mostly normal apart from several animals showing very mild periportal fatty infiltration into the parenchyma as small droplets (Fig. 5), mild bile duct proliferation persisted in Group 6 (JIB/HPD+Met) livers. In order to 'quantify' the histological findings, all liver sections were examined independantly for the presence or absence of: *a*, fatty infiltration assessed by the amount of fat seen in the hepatic cells; *b*, bile duct proliferation assessed by the number of ducts seen in the functional portal tract; and *c*, cellular infiltration. The results are tabulated in Table 2. Compared to controls the

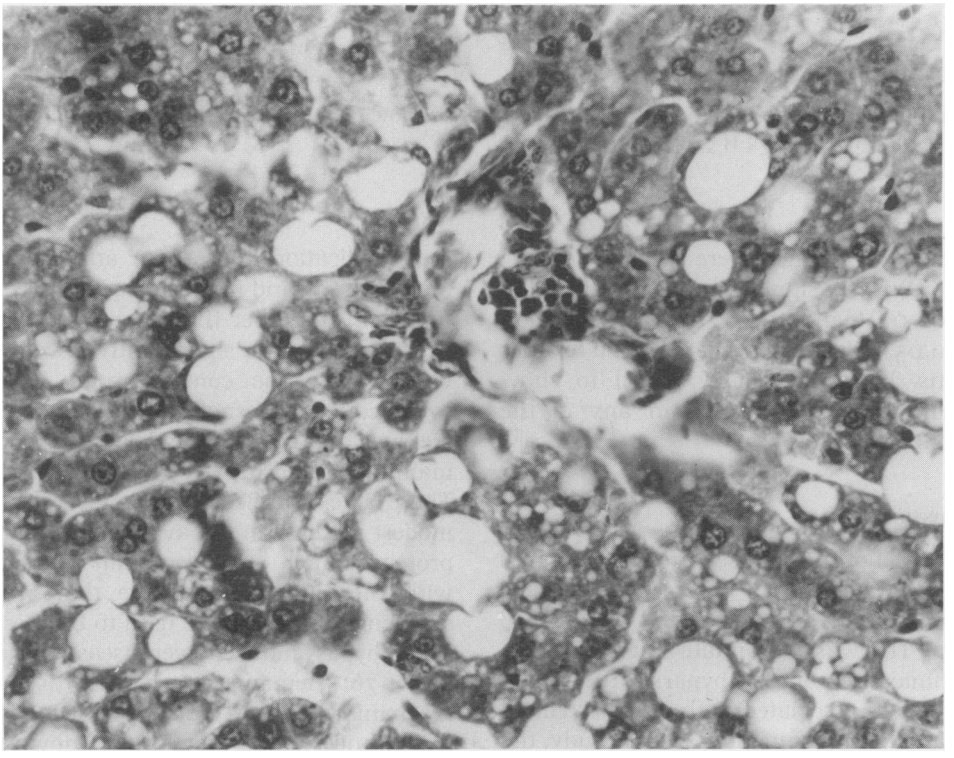

Fig. 3. Photomicrograph of a Group 3 (JIB/ND) rat liver showing severe periportal fatty infiltration. H & E, ×450.

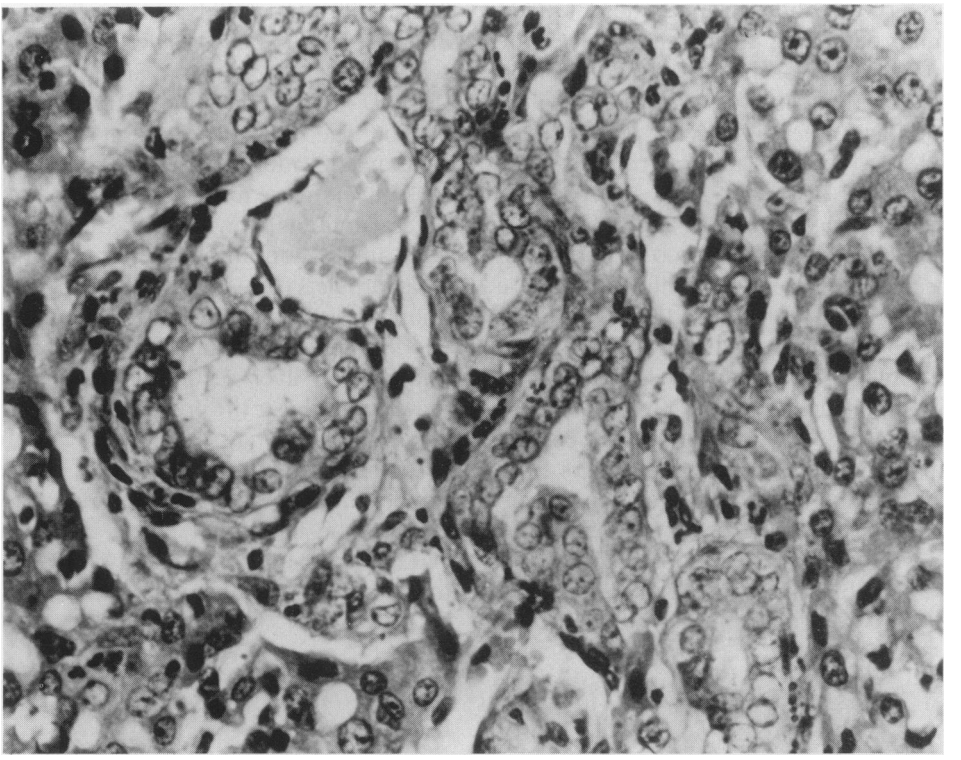

Fig. 4. Photomicrograph of a Group 3 (JIB/HPD) rat liver showing severe bile duct proliferation and very mild cellular infiltration. H & E, ×450.

most severe changes were seen in Group 3 (JIB/ND) with significant fatty infiltration ($P<0.002$) and bile duct proliferation ($P<0.05$). Histological examination of other organs, including kidney, failed to show evidence of endotoxaemia in any of the groups studied.

Discussion

The model of the JIB used in this study was more aggressive than others have selected (Hyland *et al.* 1977; Viddal & Nygaard 1977). This was appropriate as it reproduced the clinical response to bypass surgery with weight loss associated with hypoproteinaemia and liver dysfunction. In this study, the resumption of weight gain observed in those animals receiving a high protein diet was a clear indication that these animals were more healthy and in a better general condition than the other JIB rats, although they did demonstrate a significantly lower body weight than the control group. Further evidence for the benefit of the high protein diet was that the albumin levels were better maintained and the liver histology normal. These beneficial effects stem from a relatively modest increase in the available nutritional protein source, an observation in keeping with the clinical experience that protein supplementation can be used to treat established bypass induced liver disease (Ames *et al.* 1976; Heimburger *et al.* 1975). The mechanism for this protective effect of protein is uncertain, although it is known that single amino acid deficiency can induce fatty changes in the rat liver (Lyman *et al.* 1964;

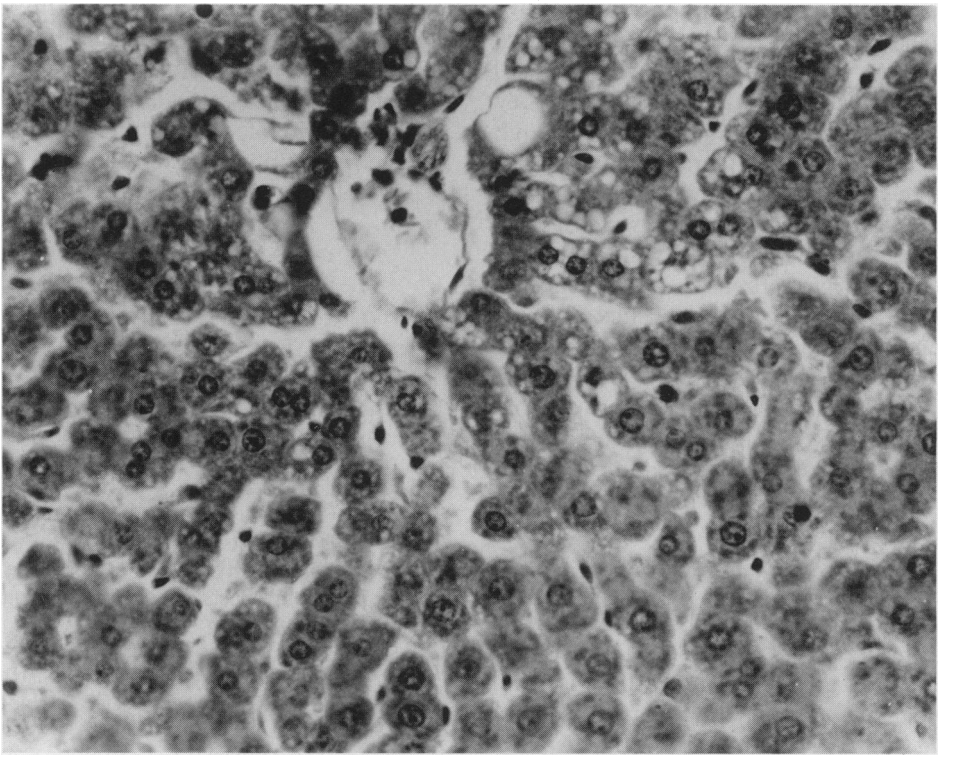

Fig. 5. Photomicrograph of a Group 4 (JIB/ND) rat liver showing mild fatty vacuoles around the periportal region penetrating into the parenchyma. H & E, ×450.

Table 2. Summary of hepatic histology in the four jejuno–ileal bypass groups

Histological features	Group 3 JIB/ND	Group 4 JIB/HPD	Group 5 JIB/ND+Met	Group 6 JIB/HPD+Met
Fatty infiltration	7/10*	0/10	1/10	0/10
Bile duct proliferation	4/10†	0/10	1/10	4/10†
Cellular infiltration	1/10	0/10	4/10†	1/10

* $P<0.002$; † $P<0.05$ (compared to controls).

Porta & Hartroft 1970; Singal *et al.* 1973). It might be that the beneficial effect of protein supplementation is based simply on the correction of a relative protein deficiency and it has been suggested that the benefit might result from an improved pancreatic exocrine function (Stock–Damgé *et al.* 1984).

The reported usefulness of antimicrobial therapy in modifying liver disease (Hollenbeck *et al.* 1975; O'Leary *et al.* 1974) remains unexplained. In this study metronidazole post-operatively in JIB rats on a normal diet gave a modest beneficial effect as shown by the body weight, plasma proteins and alkaline phosphatase parameters, but abnormal liver histology persisted with significant

cellular infiltration. Similar studies have also been reported in jejuno–ileal bypassed rats by McGouran and Maxwell (1980) and Maxwell and McGouran (1982). They reported that metronidazole, although influencing body weight and survival had no protective effect on liver function whilst ceplalexin (a cephalosporin) exerted a protective effect on liver function for 6 weeks following surgery then the emergence of resistant aerobic strains abolished the protection. They concluded that intestinal bacteria have the primary role in post-bypass hepatic dysfunction and that nutritional deficiency has an important permissive effect. In this study, when the antimicrobial therapy was used in combination with a high protein diet the results were conflicting. There appeared to be a cumulative benefit in the important parameter of plasma protein levels though no additional effect on the body weight. There was, however, significant elevation of serum alkaline phosphatase and histologically there was significant bile duct proliferation. We cannot at this time explain why metronidazole in combination with a high protein diet had this hepatic effect.

The evidence from this study suggests that both nutritional and microbiological factors are involved in the pathogenesis of bypass induced liver disease. However, one must exercise extreme caution when extrapolating experimental results from the rat into man. Obese patients coming to surgery for bypass frequently have abnormal LFTs and liver pathology even before surgery. In the rat model, the animals were healthy prior to bypass surgery, the abnormal LFTs and liver pathology being the rapid sequale to the bypass, unlike the human counterparts, where these changes are likely to have been a long term chronic process exacerbated by the operation. Nonetheless, the model reported here and by others (Hyland *et al.* 1977), although not ideal, does provide valuable clues. In the context of bypass surgery for morbid obesity, this study lends support to the proposed nutritional basis for bypass induced liver disease and the phenomenon of hypoproteinaemia. In the wider context of liver disease generally, these studies may offer an interesting model of nutritional liver disease allowing the further investigation of the inter-relationship between protein deprivation and the effects of hepatotoxins of exogenous or endogenous origin.

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